The Preparation and Use of Artificial Diets for Rearing Insects

By Brian O. C. Gardiner*

(*Continued from page* 184)

Equipment

The primary requirement is a suitable machine for mixing the medium. The one usually recommended is the Waring blender. However, any machine that is capable of thoroughly mixing a stiff substance would be suitable. In particular, many of the domestic food mixers should do the job, as also an electric drill fitted with a paint-stirring attachment or other suitable form of blade. A reasonably accurate balance is also required. The great majority of cheap ones on the market today are hopeless since they are far too inaccurate. The best is a chemical balance or the old-fashioned letter balance with pans and weights. Either of these can still be had inexpensively from local auctions and antique shops, but require searching out.

A measuring cylinder, thermometer and one or two graduated pipettes complete the equipment. A soft polythene spatula to stir and prod the medium while it is being mixed is also essential. Never use metal implements when working with blenders.

Containers

As soon as the medium has been made it should be poured into suitable containers. These should either be those in which the larvae are to feed or storage containers. For storage the diet should be poured in large slabs using any suitable large plastic box or a tin as a mould. Such slabs of diet are subsequently cut into strips for feeding to the larvae.

However, many species are best reared by being kept singly or in groups in containers in which the diet has been poured and forms a coherent slab at the bottom of the container. For this purpose glass and plastic tubes and jars of from 5 x 2.5 cm. to 1 lb. and 2 lb. jam jars are best. The warm medium is poured into the container to a depth of 1-2 cm. and the jar then slightly tipped and rolled so that about 1 cm. of the sides of the jar above the medium are coated. As soon as the medium has set, the jars or tubes should be turned upside down to protect the medium from contamination as far as possible. For storage purposes tubes can be grouped into plastic bags and jars covered with suitable lids. All diet not in current use should be refrigerated or deep frozen, depending upon its make up and circumstances.

When filling a lot of small tubes for single larvae it will be necessary to reheat the medium. This is best done by pouring it back into the agar container and keeping it in a water bath at 70°C.

The reason for not keeping the diet above this temperature which, while it would make pouring easier, is that at higher

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temperatures the ascorbic acid in particular and perhaps the antibiotic are destroyed.

Techniques

The various formulations of diets for different species have been developed to a large extent on an *ad hoc* basis but using basic knowledge of nutritional requirements. Once a diet formulation has been proved successful in the rearing of a particular species, there is of course a tendency to continue to use it. Some diets are therefore fairly specific for individual species, whilst others are of general use for a number of different species. The keeping properties of the diets also varies. It is quite possible for a larva to develop from hatching to pupation on the one container of diet. In general, however, better results are obtained when — and it is certainly advisable to do this — the diet is changed every week. Some diets can be stored almost indefinitely at −25°C., whilst others have their gel structure destroyed at this temperature and can only be kept for a few weeks at 4°C.

Basically, all the artificial diets consist of some sort of plant protein, derived from either beans or wheatgerm together with casein and sugar. A comparatively high proportion of Vitamin C is also necessary. Most of the other ingredients used are for binding and keeping off the bacteria and fungi for which the diet is an ideal medium for growth. The methods of making up the diets also varies, but provided certain precautions are followed I have found from experience that it does not seem to matter. Essentially, ascorbic acid and antibiotics are unstable at high temperature and must therefore be added last, after the boiled agar (which should also be cooled a little before hand) is mixed in with the rest of the diet. The various ingredients are sometimes added separately; at other times they are premixed and an aliquot then taken. Various additives may be put in either as solids or as solutions. The actual method to use will depend partly on the resources of the individual and partly on the quantities of diet that it is required to make on a regular basis.

Before making up a diet, one must also consider the habits of the insect to be reared. Is it a surface feeder such as *Pieris brassicae,* or an edge feeder like *Manduca sexta*? Surface feeders are best reared in tubes and jars with the diet poured into them to a depth of 1-2 cm. Edge feeders are best in rectangular plastic boxes with the diet supplied in cut strips. These should not be thinner than 0.5 cm. in section or they may dry out and harden too quickly. The bottom of the boxes should be lined with Kleenex tissue and a piece of expanded aluminium mesh or other metal gauze cut to fit inside the box upon which the strips of diet rest. This allows the frass to fall clear of the diet. In this connexion also, all tubes and jars of diet should be kept on their sides in order to allow the frass to fall clear of the feeding surface. Containers containing newly hatched and small larvae should be closed with metal foil or polythene for the first few days.

After the larvae have grown a bit (which in fact means they can cope with a slightly drier surface), paper closures should be substituted. Large larvae require good ventilation and open mesh closures will then be needed.

It is a mistake to allow larvae to pupate in the artificial diet. Butterfly larvae will normally leave the diet and attach themselves to the side of the container, when all have done this any remaining diet should be removed. Species which normally pupate in the soil require to be removed when ready. Since all larvae change colour and become migratory at this time they are easy to pick out and several methods of pupation have been used. A simple one is to place them in a small container with a Kleenex tissue and well fitting lid. Always then keep them in the dark.

One a diet is made up, it will commence to dry up from the surface downwards. In spite of its compliment of fungicides and antibiotics, various fungal spores and bacteria will settle on it and try to multiply. Provided the surface is being browsed by sufficient larvae none of these events need give any trouble. One of the secrets of success, so to speak, in the use of artificial diets, is to get the balance right in having enough insects in a given size of container to eat the surface of the diet faster than it can dry out, or pathogens of the diet establish themselves. As a guide, some 20 *P. brassicae* in a 5 x 2.5 cm. tube or 100 in a 1 lb. jam jar, and 20 *M. sexta* larvae on a 15 cm. strip of diet. As the larvae grow of course these numbers should be reduced, and large larvae may even be reared singly. Naturally gregarious species, however, I would always recommend to keep in groups. Species such as *Cosmia trapezina,* which are normally cannabilistic, should naturally enough always be kept singly, but there is no doubt that they give more trouble and need greater care in looking after.

I have already stated that it has nearly always been found that changing the diet weekly gives the best results.

When the larvae are small, there is no need to give them any further attention at all. By the time they reach the final instar, those that are being kept in groups may need the frass produced removing, and may also need additional food. With larvae being kept singly, no attention is required until pupation time. Most species thrive best on the diets when they are kept at a fairly constant temperature of from 20-25°C. and a relative humidity of between 50-70.

Diet formulae

All the diet combinations now given are based on well tried formulae and have been in use for several years. To some extent they are interchangeable and little or no difference in size, longevity or fecundity can be found when species are reared on the different formulae. What would be of interest would be to investigate the full range of insects—not just lepidoptera—that can be reared on each. In general, polyphytophagous species are more accommodating as regards acceptance of diets than are host specific feeders. For the

sake of convenience, all quantities have been calculated to make up a batch of approximately 750 ml., which is the amount that can conveniently be made in the usual types of kitchen blender.

SHOREY'S MEDIUM

Developed by Dr. H. H. Shorey in California, U.S.A. A fairly simple medium requiring few ingredients and has been shown to be suitable for the following Noctuid larvae:— White speck (*Leucania unipuncta*); Pearly underwing (*Peridroma saucia*); Ni moth (*Plusia ni*); Bean leaf skeletonizer (*Autoplusia egena*) (but adults laid no viable eggs); Small mottled willow (*Laphygma exigua*); American brocade (*Prodenia ornithogalli*); Alfalfa looper (*Autographa californica*); Bollworm (*Heliothis zea*); Tobacco budworm (*H. virescens*); Cabbage moth (*Mamestra brassicae*). Larvae reared on this medium have been kept at 27°C. and under these conditions complete their development in 14-21 days and feed throughout on the same batch of medium and pupate in their jars. About 15 larvae per 1 lb. jar containing 2.5 cm. depth of medium. When kept at a lower temperature, the medium should be changed half-way through the life-cycle, and in any case at 14 days. The large *Heliothis* must also be separated for they are cannibals.

This medium, although simpler, takes longer to prepare and is not, in our opinion as versatile as the next one. Survival rate, egg to adult, rarely exceeds 50%, while Ignoffo's medium (see below) has given nearly 100%. It is not as acceptable to such a wide range of species, being of no use for Cabbage whites and poor for Arctiids. It does, however, seem to last longer and will, no doubt, prove to be acceptable to a larger range of Noctuids than those so far tried on it. The addition of about 1.5% of dried cabbage leaf powder improves its acceptability to Garden tiger larvae and improves the performance of Cabbage moth. It has the advantage of not requiring the vitamin solution needed by other diets.

The medium consists of the following ingredients:— 214 gms. of soaked Haricot beans; 32 gms. of dried Brewer's yeast; 3.2 gms. of Ascorbic acid; 2.0 gms. of Methyl para hydroxbenzoate; 1.0 gms. of Sorbic acid; 2.0 ml. of Formaldehyde (40%); 12.8 gms. of Agar; 640 ml. of Water.

To prepare the medium, dried Haricot beans are first soaked overnight at 20-25°C. They are then brought to the boil, drained and weighed out. They are then added to one half of the water and must be thoroughly pulped using a suitable blender, as already mentioned. The other ingredients are then mixed in, with the exception of the agar. This is boiled with the other half of the water, using a water bath as already described. It must now be allowed to cool to 70°C. when it should be mixed in to complete the preparation of the medium, which is now poured out into the required containers and in a few minutes has set and is ready for use. This diet does not apparently keep, even if deep frozen, and should therefore be made up fresh when needed.

(*To be concluded*)